
# MicroFluidic Molecular Systems

## MICROFLUMES

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## MicroFluidic Molecular Systems (MicroFlumes) Vision


A SINGLE PROGRAMMABLE MACHINE that performs 100s of fluid-based process sequences to meet dynamic requirements in Chem/Bio Analysis & Synthesis

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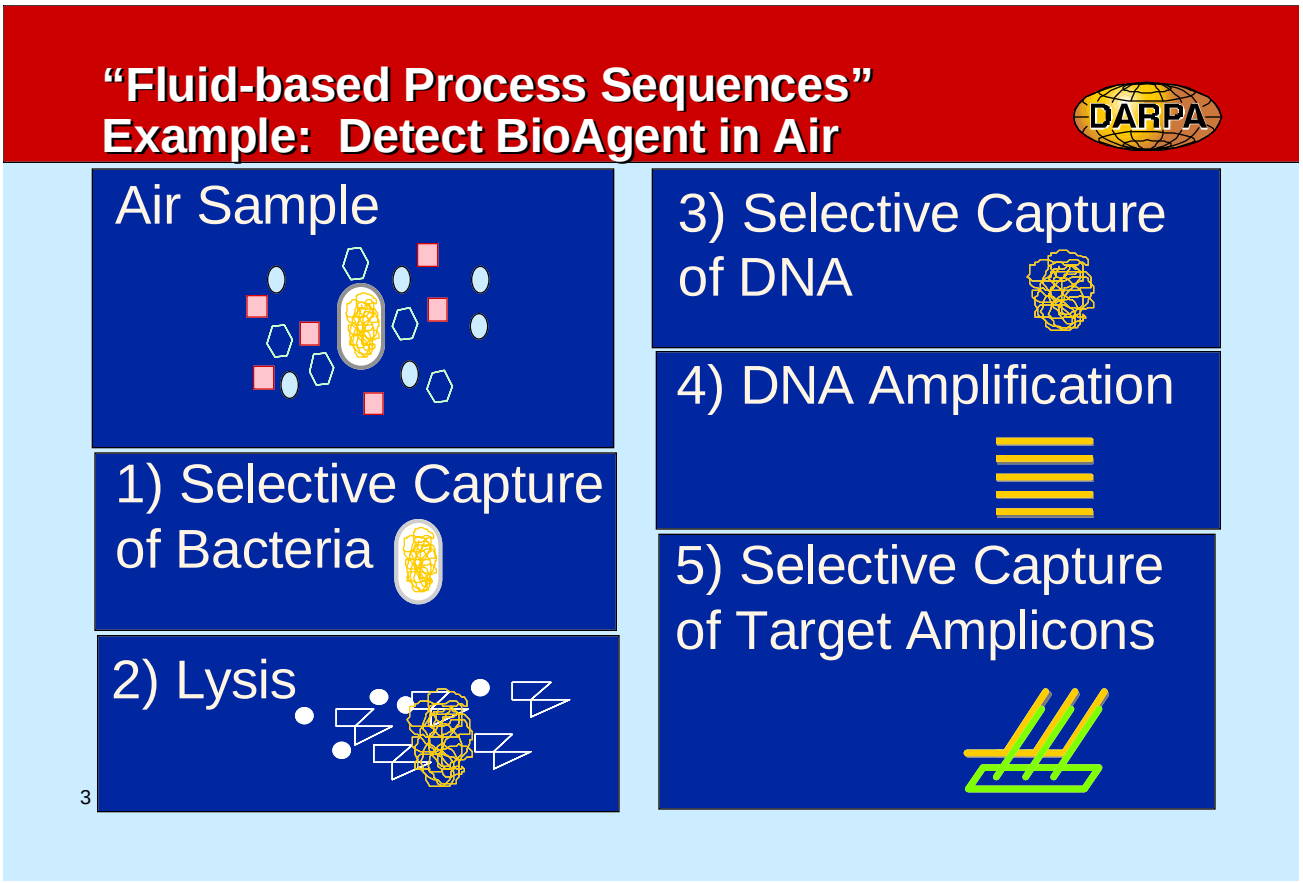
The Vision of the MicroFlumes Program is a single programmable machine that performs 100s of fluid-based process sequences to meet dynamic requirements in Chem/Bio Analysis and Synthesis.




A"fluid-based process sequence" is a combination of steps such as fluid metering, mixing, separation, and reaction. In this example shown in this slide, the task is to detect a hazardous bacterial bioagent in air. One way to accomplish this task using DNA Assay is shown. Each block in the figure is a fluid-based process sequence.

We start with an air sample. The target bacterium is shown in yellow. The air sample is collected in a liquid medium for further processing.

The first step is to selectively capture the bacteria of interest — to separate it from the other particles in the air sample for further processing.

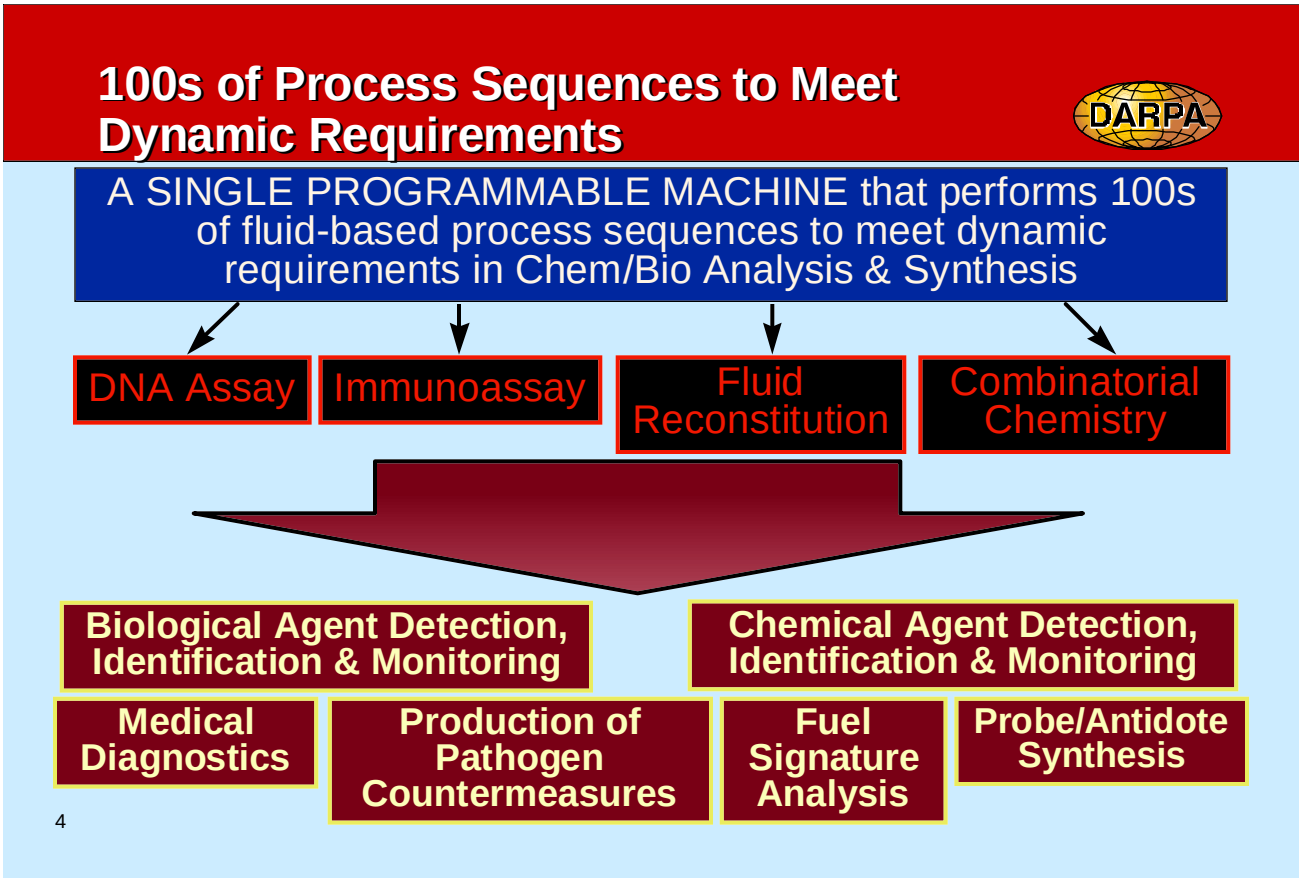
The second step is lysis — breaking open the cell wall of the bacteria.

The third step is selective DNA capture — separating the DNA that is needed for analysis from the other cellular constituents.

Fourth, the target DNA is amplified or copied.

Finally, the target amplicons — or amplified copies of the target DNA — are captured. In this step, there is a probe containing a complementary DNA sequence to the target. This is shown here in green. The probe binds to the amplified DNA, shown in yellow, only if there is a complementary match.

Each of these blocks is a "fluid-based process sequence." The DNA amplification step, which is usually repeated 20-30 times to provide enough sample for detection, requires the addition of 3-4 chemical reagents as well as several wash sequences.




The MicroFlumes Program is driven toward a single programmable machine **that performs 100s** of such fluid-based process sequences to meet dynamic requirements in Chem/Bio Analysis and Synthesis.

“Fluid-based process sequences,” as shown in the black boxes, can include processes to accomplish DNA Assay (as shown in the previous slide), antibody-based immunoassay, combinatorial chemistry, fluid reconstitution and a host of others, such as cellular monitoring. This slide is meant to provide examples, not to be an exhaustive list.

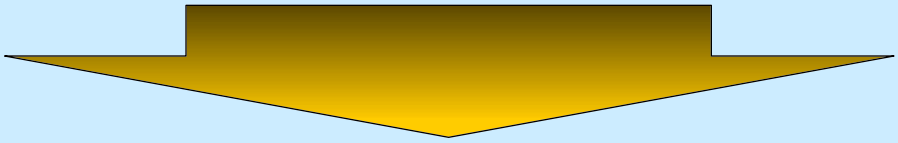
Some examples of Military Applications of Chem/Bio Analysis & Synthesis are shown in the red boxes. They include biological and chemical agent detection, identification and monitoring, medical diagnostics, fuel signature analysis, environmental monitoring, and field-based synthesis .... of detection probes, antidotes and blood or chemically based pathogen countermeasures.

We’re going to achieve this vision by building on the two functional lessons learned in VLSI or Very Large Scale Integrated electronics: (1) Components microfabricated with integrated control allow manageable complexity. (2) Complex microfabricated systems with integrated control enable new ways to accomplish tasks that have no macroscale parallel.

The next key word in the Program Objective that I’d like to define further is “programmable.”

## Programmable Process Flow

- Store and execute a range of fluid-based process sequences or “programs”
- Control program execution by local or remote re-tasking
- Operate for extended intervals (execute >1000 “programs”) without human intervention



**Customization During & After Deployment to Meet Evolving Requirements**

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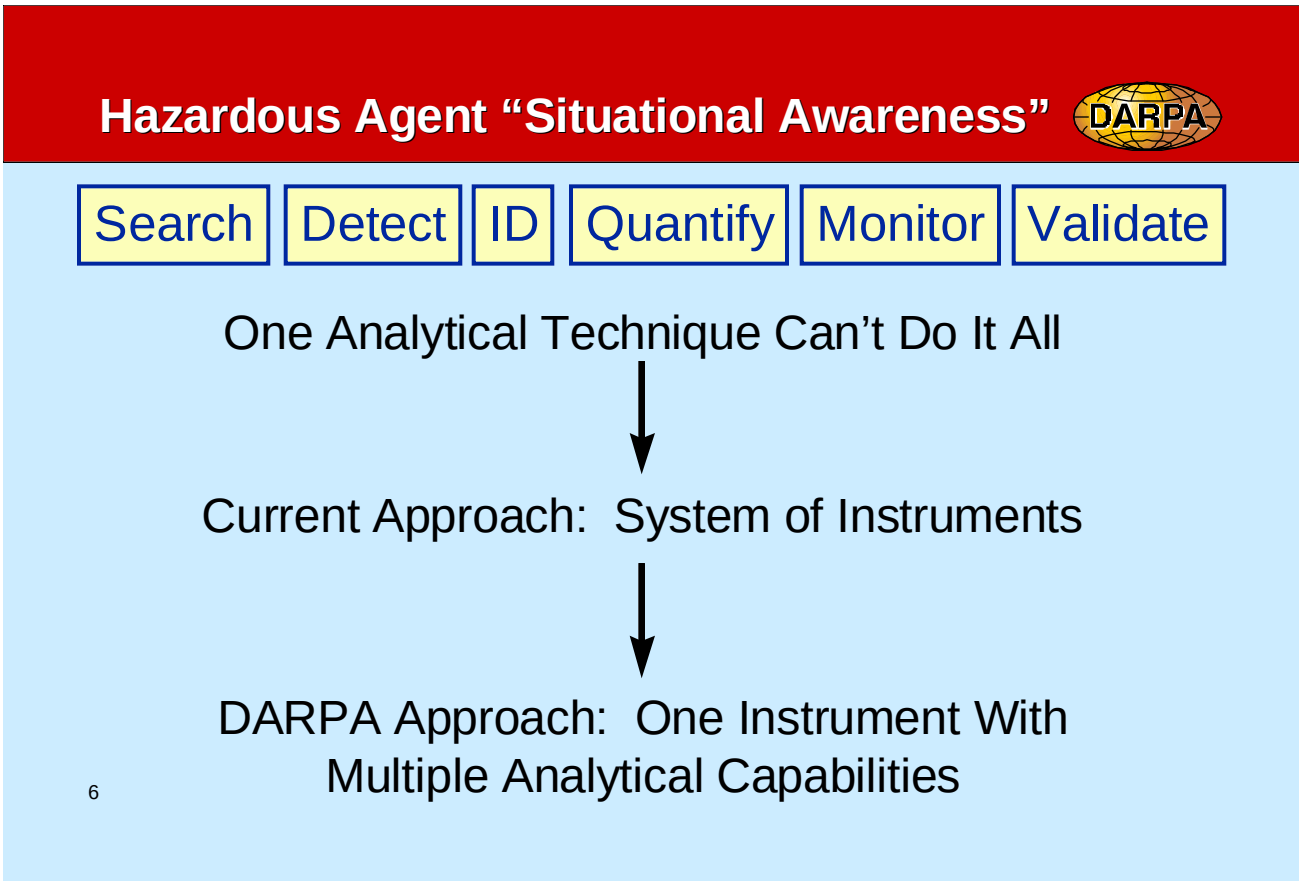
Programmable means

- The capability to **Store** a range of fluid-based process sequences or “programs”;
- The capability to control program execution — to specify which program should be running at any given moment and to customize the specific analysis and synthesis targets — through an electronic interface to allow local or remote retasking; and
- The capability to operate for extended intervals (execute >1000 “programs”) without human intervention — which means storing the reagents needed for a wide range of tests.

Bringing these capabilities together in a MicroFluidic System will allow customized performance during and after deployment: (1) to switch from one application to another, such as detection of hazards in aerosol samples from the battlefield one moment and detection of infection in blood samples the next; and (2) to move through a range of complementary sample preparation and analysis methods when needed for specific identification and verification.




To use the task of hazardous agent detection as an example once again and to provide awareness of the complete spectrum of hazardous agents in a complex and dynamic battlefield environment require several subtasks — search, detect, identify, quantify, monitor and validate.

Each of the tasks has its own specific requirements. Some tasks require very high sensitivity — the capability to find a target at very low concentrations; some require high specificity — the capability to distinguish between similar targets to identify the specific agent present; some tasks require accuracy — not just knowing that the target agent is present but being able to quantify the level of that threat; and some require redundancy, the capability to perform a repeat test on the same sample in order to confirm or validate the threat.

What we have found is that one analytical technique can't do it all.

The current approach is a system of instruments. The DARPA approach is to provide multiple analytical capabilities in one instrument.

This is quite a challenge, given that today most bio/chemical analysis and synthesis involve the manual labor of trained technicians in a laboratory setting.

## Autonomous MicroFluidic Detection System


- Programmable, Multifunction capability in one unit
- On-board reagents for hundreds of tests: long-term use, validation/verification, and generation of "all-clear"

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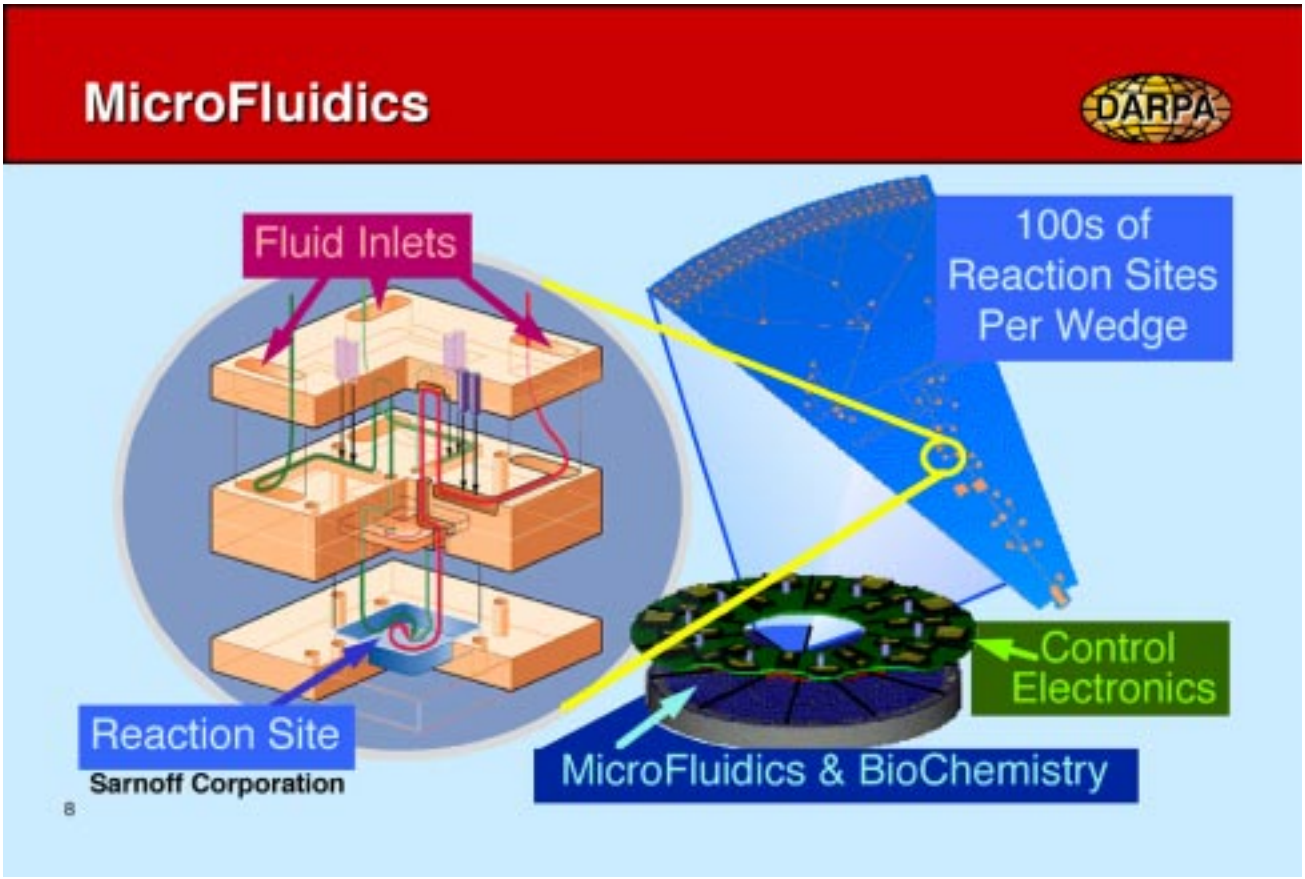
On the right side of this slide is a conceptual drawing of an autonomous, microfluidic system for the detection of hazardous bio/chem agents, from Sarnoff Corporation, one of the contractors in the DARPA MicroFlumes Program.

On the left is a HMMV, one type of vehicle that is used today to hold instruments and field crews for field-based assays for hazardous bioagents.

The goal of the Sarnoff effort is to provide 100x the capability of the HMMV system in a machine with the size and weight profile of a notebook computer, that:

- is truly stand alone, with all sample handling and prep steps integrated and automated, starting with the collection of an aerosol sample and including sample affinity capture to separate bacteria from other material found in the concentrated air sample, selective capture of the bacteria of interest, lysis to break the cells open, separation of DNA from other cellular components, selective amplification of target DNA, capture of amplified DNA, and DNA-based identification;
- is digitally controlled for real-time or remote retasking; and
- contains on-board reagents for 100s of tests for long-term use and validation/verification.




This slide shows the current phase of that project.

On the left is an expanded view of a millimeter-sized, multilevel fluidic processing network.

The network moves fluids through between 3 and 5 layers of fluidic interconnect to achieve the type of integrated functionality and density we have come to rely on in multilevel electronic circuit wiring.

Large networks of fluidic channels are integrated onto pie-shaped wedges, shown here in blue.

The pie-shaped wedges are integrated with control electronics, shown here in green, to form a 5″

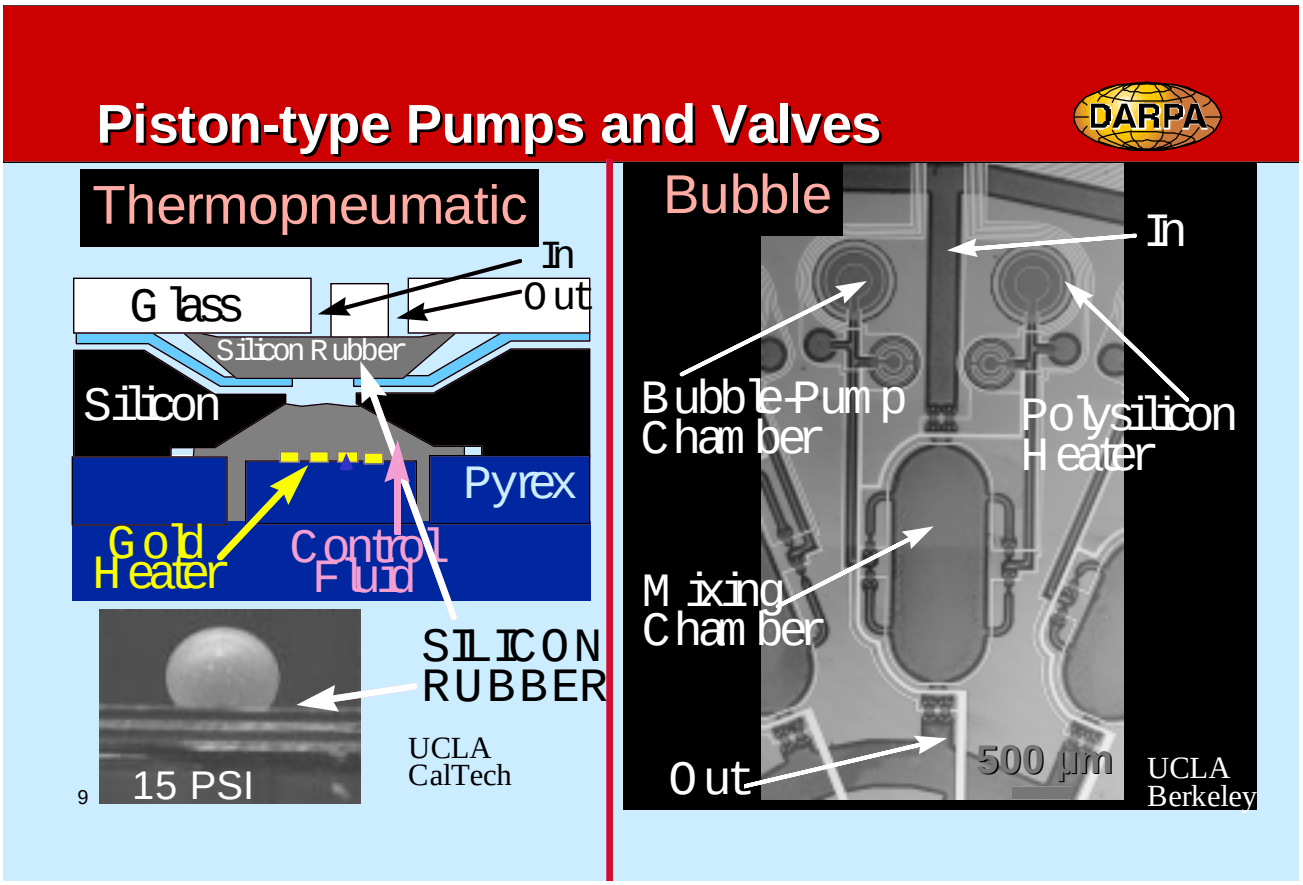
-diameter donut.

The capability to control the transport and reaction of fluids in a complex network of microfabricated channels is the key enabling technology to achieve the vision shown in the previous slides.

The microfluidic transport strategy used in this project is electrokinetic — there are no specific pump components and no membrane or piston-like component to move the fluids. Instead, the fluid movement is controlled by applied voltages and surface charge. Valving, Mixing, and Load and Inject functions occur by changing the applied voltages at the end of each channel.

Because there is no specific “pump” component, I refer to this type of fluid movement as “Virtual” Pumping and Valving. Channels and junctions can be made into programmable pumps, valves and processing reservoirs through applied electronic control.

In addition to Electrokinetic or “Virtual” fluidic strategies, there are a number of techniques to micromachine “piston-type” microfluidic pumps and valves.




This slide shows two “piston-type” pumps. These are examples of the types of techniques developed in the MicroFlumes Program.

On the left is a Thermopneumatic pump, developed as part of a MicroFlumes contract at the University of California, Los Angeles, and the California Institute of Technology. In this micromachined device, gold-heating elements are used to control the expansion of a control gas that then causes the deflection of a silicon rubber membrane. Pumping action is achieved as a result of the membrane deflection.

On the right is a Thermocapillary Pump (“bubble pump”) developed as part of a MicroFlumes contract at the University of California, Berkeley. A Micromachined polysilicon heater, shown in the circles toward the top of the diagram, provides heat, causing the formation of a bubble. The formation of the bubble at the top of the channel displaces fluid at the bottom of the channel, effecting a piston-type pumping action.

## MicroFluidics Challenges

- clogging
- bio/chem compatibility of surfaces
- transport of complex and varied fluids
- control adjustments needed as a function of fluid
- leakage
- flow/volume control
- precision
- accuracy
- response time
- max operating speed
- power
- dead volume
- contamination of the fluid
- keeping fluids “in”and “out”
- diffusion through materials over time
- control of surface tension
- bubbles
- small particle trapping
- fatigue
- wear
- instruments to characterize/visualize flow
- failure analysis
- real world interface

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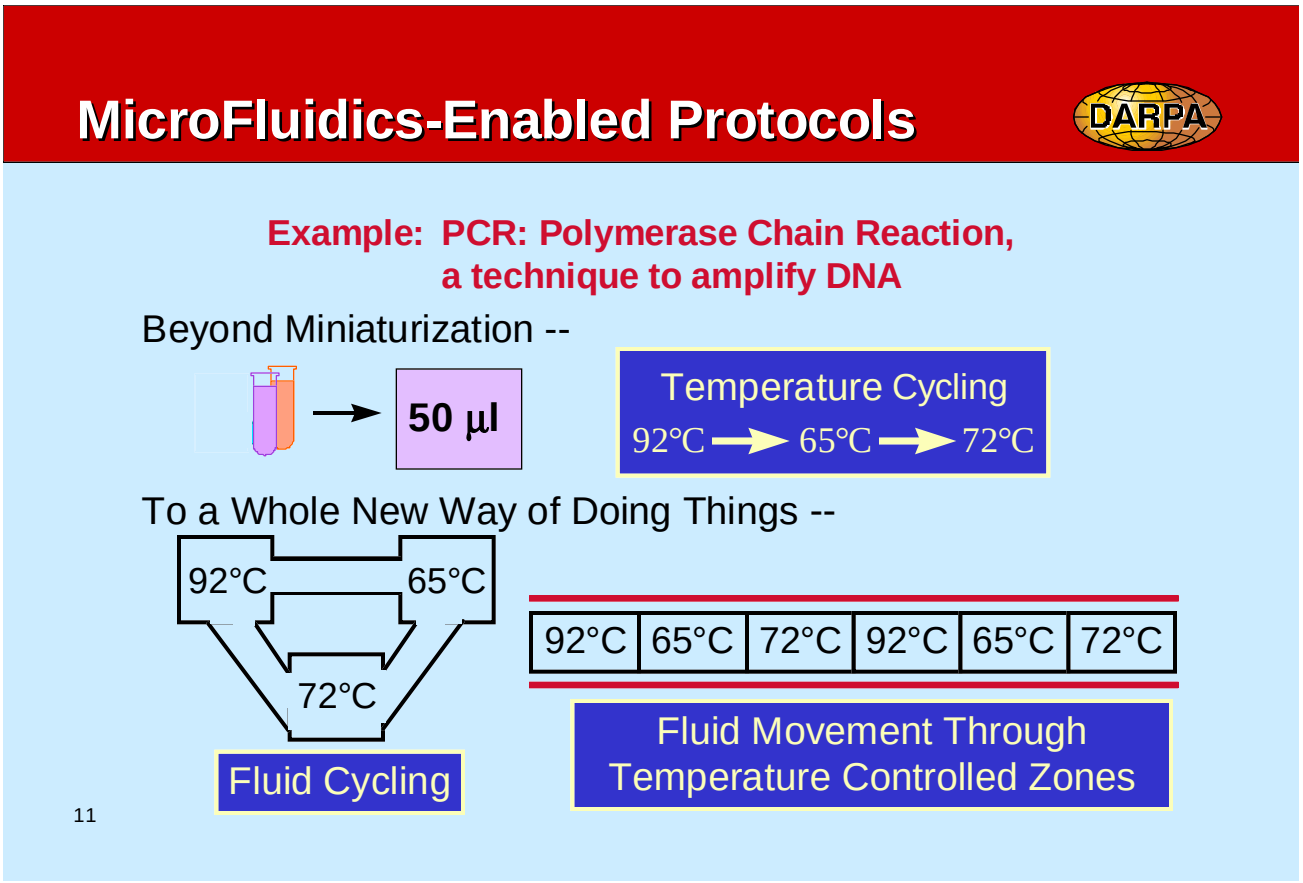
Each microfluidic strategy has advantages and drawbacks when considered against the list of challenge areas shown here. In order for programmable microfluidic systems to become a reality, each of these challenges must be addressed and solved.

The development “wetware” — microfabricated structures for controlled fluidic transport — is a primary area of focus in the MicroFlumes Program.




But MicroFlumes is more than the development of "Wetware" — a second key thrust is the development of microscale-enabled protocols.

Here is an illustration of this concept involving PCR. PCR is the Polymerase Chain Reaction used to amplify DNA. PCR is a temperature cycling process — you put a single DNA molecule, along with the appropriate reagents, in a test tube, and each time the temperature is cycled from 92 degC to 65 degC to 72 degC, a copy of the DNA is made. There are tabletop instruments available from a number of manufacturers to do PCR.

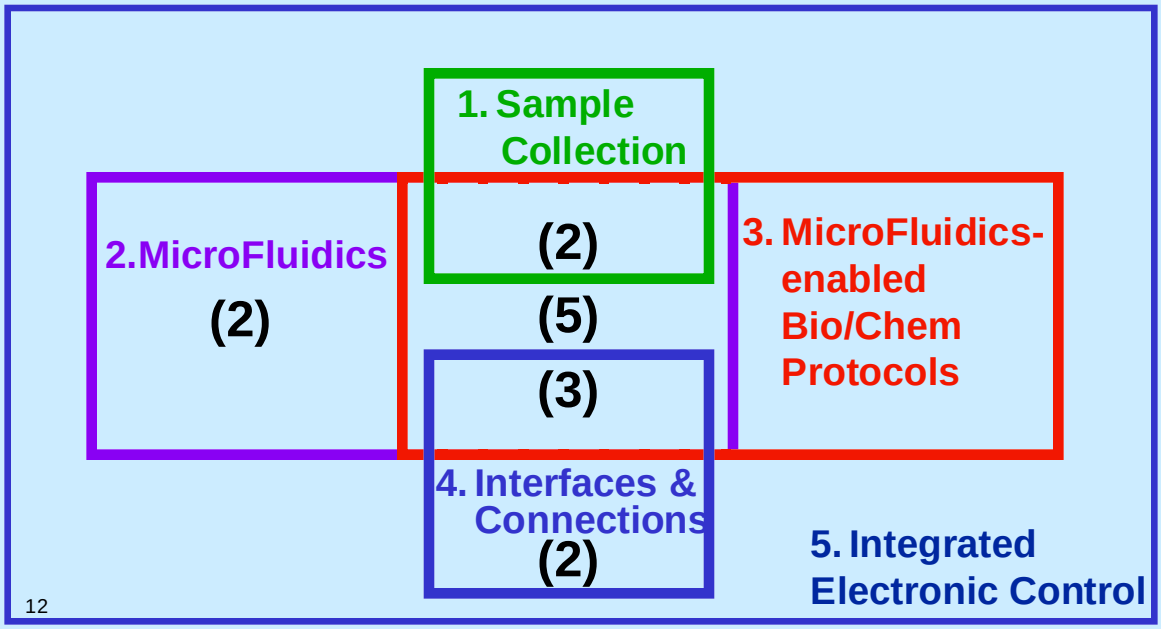
In 1994, DARPA funded the development of a Miniature Analytical Thermal Cycling Instrument to demonstrate the PCR process in 50 µl micromachined reservoirs. The effort was a success — PCR works in the microfabricated structure and is 10 x faster than commercial instruments, largely because it is easier to control temperature in smaller regions — you can control temperature much better in a 50 µl reservoir than in a test tube.

But now, what if, instead of making the test tube smaller, we get rid of temperature cycling all together and replace it with fluidic cycling? That is, take three microreservoirs and hold them at the right temperatures. Then cycle the fluid from one place to another instead of holding the fluid in the same place and cycling the temperature. This is a microfluidics-enabled technique — you couldn't ask a technician in a laboratory, or even a robot, to physically move a fluid from place to another in 30 second time windows, but with channels and pumps microfabricated on a chip, along with control electronics, this kind of idea becomes feasible.

Or, how about if, instead of three reservoirs, we have a long channel and we have a series of zones, each of the appropriate heat? Then we can just push the fluid through the channel to amplify — this will be really FAST! Or, can we get away from temperature altogether and somehow get to isothermal, or constant temperature, amplification? Because even though temperature cycling works well in the laboratory environment, it may be difficult to bring this into the field for portability and in situ operation.

Microscale-enabled protocols are about accomplishing the task in a whole new way — like binary math. You wouldn't do it that way on paper, but it makes sense for a computer.

# Target Investments




This slide shows the framework for the 14 contracts already in place in the MicroFlumes Program.

Two contracts focus solely on novel microfluidic pumping strategies — these are to the left in the purple box. Two contracts focus solely on bio/chem compatibility of interfaces and micro-macro scale interconnect; these are the two in the blue box toward the bottom.

The majority of the contracts fall in the middle area, combining the development of microfluidics with the development of microfluidics-enabled protocols. These are the 10 where the purple and red boxes overlap. Of these 10, 3 *also focus* on interfaces and connections, and 2 also focus on automating the sample collection process. Half of these efforts are aimed at challenges in the detection of chem/bio hazards. The others involve synthesis, fluid reconstitution and other types of bioanalysis.

A focus on integrated electronic control is part of all of these efforts.

## Next Phase

BAA 97-39 in Progress

- ➢ $25M over three years
- ➢ Proposals due November 7, 1997

Areas of Interest:

1) Systems-driven development of microfluidic components and microfluidics-enabled processes
2) Design Tools for the Development of Mixed Technology Systems that include MicroFluidics

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This slide shows the Next Phase of the Program. BAA 97-39 is currently open, with proposals due November 7, 1997.

Multiple awards are expected with a planned investment of $25M over three years. This is a combined BAA from the MicroFlumes Program and the Design for Mixed Technology Integration Program, with two areas of interest as shown here.

## Beyond Miniaturization


MicroFlumes:
To fundamentally alter the way we perform analysis and synthesis

Through New Developments in:

- MicroFluidics, and
- Microscale-enabled protocols

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Finally, here is the one idea to carry away from this presentation when you think about MicroFluidic Systems. The focus of this program is BEYOND miniaturization.

MicroFlumes will fundamentally alter the way we perform analysis and synthesis tasks, just as computers have fundamentally altered the way we perform arithmetic and logical operations.

In the 1920s and 30s when complex mathematical calculations were performed by groups of trained individuals known as “computers,” the notion of working with strings of binary numbers or any of the modern advances in software and operating systems would simply not make sense.

We must go beyond the miniaturization of laboratory processes to create systems that are more than what we can envision today as a “lab-on-a-chip.” In 20 years, the idea of calling a microfluidic system a “lab-on-a-chip” will seem as strange as thinking of a computer as an automated slide rule.

The way we’re going to get there is to focus on microfluidics and microscale-enabled protocols — to focus both on components and on new modes of operation.

These are the core premises of VLSI that drive MicroFlumes just as they drove computer development. Components microfabricated with integrated control to achieve manageable complexity AND the new protocols that these complex systems enable — new ways to accomplish tasks — that have no macroscale parallel.

Thank you.